

To the Advisory Committee to the Director and the Working Group for Human Embryonic Stem Cell Eligibility Review:

By this submission, Harvard University requests that NIH approve for use with federal funds 28 human embryonic stem cell (hESC) lines derived at Harvard. We submit these materials to the Advisory Committee to the Director and the Working Group for Human Embryonic Stem Cell Eligibility Review for approval.

Introduction

Harvard's naming convention for the hESC lines it has developed is to identify the lines with the acronym HUES, which stands for Human Embryonic Stem Cells, and then to number the lines sequentially. HUES 1-28 were all derived from embryos donated before July 7, 2009 and, therefore, are being submitted under the criteria set forth in section II(B) of the National Institutes of Health Guidelines for Human Stem Cell research (the "NIH Guidelines"), 74 Fed. Reg. 32170 (July 7, 2009). This application demonstrates that these cell lines meet the criteria set forth in section II(B).

For the sake of context, and to foreshadow future Harvard submissions with respect to other HUES lines, we provide here some general information about Harvard's stem cell program. Harvard researchers so far have derived 82 hESC lines, 65 of which have been the subject of scientific publications. Seventy of the lines are simply known as HUES lines. These lines, including HUES 1-28, were derived from *in vitro* fertilization (IVF) embryos donated by couples who had completed their fertility treatments. Certain other Harvard lines were donated by couples who had undergone IVF for purposes of pre-implantation genetic diagnosis. We use the acronym "HUES PGD" to identify these hESC lines. HUES PGD lines carry genes for specific diseases.

HUES 1-28 are Harvard's oldest lines and were all derived under a single IRB-approved protocol and informed consent form. A subset within this group, HUES 1-17, have been distributed by Harvard's Office of Technology Development to more than 200 institutions worldwide. We believe these lines may be in more research labs than lines created by any other single institution.¹

In support of our request for the approval of HUES 1-28 for eligibility for NIH funding, we have attached the following documents:

1. Consent to Donate Human Embryos and Embryonic Cells for Research ("Consent Form")
2. Request for Approval of Human Subjects Research (the "Protocol"), submitted in March 2000.
3. Proposal for the Generation of Human Embryonic Stem Cells (the "Proposal"), dating from October 2000.
4. Harvard IRB² contingent approval letter, dated December 19, 2000.

¹ Since 2004, Harvard has distributed more hESC lines per year than WiCell (see McCormick, JB, Owen-Smith, J, Scott, CT. Distribution of human embryonic stem cell lines: who, when, and where. *Cell Stem Cell*. Feb 6, 2009; 4(2):107-110), despite the fact that the WiCell lines have been eligible for NIH funding, while the HUES lines have not. According to the California Institute of Regenerative Medicine data on hESC Utilization for Disease Research that was posted on CIRM's website in May 2009, HUES 1-17 are being used by researchers to study ALS, Alzheimer's disease, Huntington's disease, Parkinson's disease, spinal injury, heart disease, hematopoietic conditions, cancers, infertility and musculoskeletal illnesses. These lines also are considered the "gold standard" for the comparison of new materials with claimed pluripotent properties (Silva SS, Rountree RK, Mekhoubad S, Lee JT. X-chromosome inactivation and epigenetic fluidity in human embryonic stem cells. *Proc Natl Acad Sci USA*. 2008 March 25; 105(12):4820-5).

² Harvard University has three IRBs. The University Area IRB, also known as the Committee on the Use of Human Subjects, reviewed the Protocol. It will be referred to herein as the IRB.

5. Letter from the Commonwealth of Massachusetts, Middlesex District Attorney's Office, dated December 19, 2001.
6. Harvard Stem Cell Research Committee letter, dated March 27, 2002.
7. Harvard IRB statement regarding distribution of HUES line 1-28, dated July 17, 2008

We will refer to these documents throughout this submission. We have annotated the Consent Form, Protocol, and Proposal to make reference to specific criteria in the NIH Guidelines.

Though we have attached supporting documentation to this request, we briefly describe for you here the history of oversight that has been exercised over the Protocol that led to the development of HUES 1-28.

Background and History

The Protocol and Consent Form initially underwent three review processes. First, the IRB reviewed the Protocol and Consent Form under 45 C.F.R. pt. 46 even though the research was not federally funded. The IRB also required that the study meet what were then commonly known as the "Clinton Guidelines" for human embryonic stem cell research.³ The IRB contingently approved the Protocol on December 19, 2000 (Document 4), and all the contingencies were satisfied by January 9, 2001. A second review was required by a Massachusetts law (M.G.L. c.112, §12J) that prohibits certain scientific research on embryos. Under that law, the Protocol was approved by the Middlesex County District Attorney's Office on December 19, 2001, Document 5. The third review process was one created by Harvard specifically to oversee hESC research. This review body, selected by the University's Provost, was known as Harvard's Stem Cell Research Committee ("SCRC") and was chaired by Richard Losick, Maria Moors Cabot Professor of Biology. The SCRC's review pre-dated the National Academy of Sciences' Guidelines for Human Embryonic Stem Cell Research, but addressed the same sort of ethical concerns later articulated by the NAS. The SCRC's approval of the *in vitro* (derivation) aspects of the Protocol occurred on December 21, 2001. Document 6.

The Protocol originally was proposed as a collaboration among two professors at Harvard University in the Department of Molecular and Cellular Biology,⁴ Douglas Melton, Thomas Dudley Cabot Professor of the Natural Sciences, who was the Principal Investigator (PI), and Andrew McMahon, Frank B. Baird Jr. Professor of Science, and a third person who was the Scientific Director, but not a clinician, at a Boston-area IVF clinic (the "Collaborating IVF Clinic"). Over time, potential donors from outside the immediate Boston-area expressed interest in donating their frozen IVF embryos to Harvard's research program and they were told to contact the Collaborating IVF Clinic. All donors regardless of where they had received fertility care continued to be consented by the Collaborating IVF Clinic staff and all donated frozen embryos were held at the Collaborating IVF Clinic until they were ready to be transported to Harvard for the research. Under the Protocol, Harvard did not receive from the Collaborating IVF Clinic any identifying information about the donors, including whether the donors were patients of the Collaborating IVF Clinic or other IVF clinics.

³ Nat'l Institute of Health, Guidelines for Research Using Human Pluripotent Stem Cells, 65 Fed. Reg. 51976 (Aug. 25, 2000).

⁴ These two investigators are now members of Harvard's Department of Stem Cell and Regenerative Biology.

HUES 1-28 meet the requirements of section II(B)

Section II(B) of the NIH Guidelines has two requirements. The NIH Guidelines state that eligible hESC lines must be derived from embryos “(1) That were created using in vitro fertilization for reproductive purposes and were no longer needed for this purpose; and (2) that were donated by donor(s) who gave voluntary written consent for the human embryos to be used for research purposes.” NIH Guidelines, 74 Fed. Reg. at 32175. HUES 1-28 meet these criteria as documented by the Protocol, Proposal,⁵ and Consent Form. As noted in the Protocol, the subjects would be “prospective parents” who had sought IVF treatment. Document 2, at 2. As further explained in the Protocol, “[t]he embryos are obtained only after the subjects and their physician have agreed that the embryos are no longer needed for treatment of their infertility.” *Id.* at 3. As further explained in the Proposal, “Frozen embryos used in the derivation procedure are excess, not required for clinical infertility procedures and were frozen and stored prior to the decision to donate the frozen embryos to research.” *See* Document 3 at 4. The Consent Form (Document 1) demonstrates that donors provided their voluntary and written informed consent to donate embryos to research that was specifically described as intending to create hESC lines.

Section II(B) also provides that the Working Group will consider three additional criteria concerning the informed consent process. Whether “the donors(s) were: (1) Informed of other available options pertaining to the use of the embryos; (2) offered any inducements for the donation of the embryos; and (3) informed about what would happen to the embryos after the donation for research.” NIH Guidelines, 74 Fed. Reg. at 32175. Harvard’s informed consent process for the derivation of HUES 1-28 also meets these criteria. The Consent Form informed donors that the research program was an alternative to the decision to have their frozen embryos discarded. Document 1 at 2. Couples who were still considering using the embryos themselves or who intended to continue to store their frozen embryos were not offered the option of this Protocol. Further, as explained in the Proposal there was “no inducement, financial or otherwise, provided to the donors of the embryos.” Document 3 at 4. *See also* the Protocol which states, in response to a question as to whether subjects will benefit by the research, that the only benefit is the indirect one of “advancing studies on human embryonic development.” Document 2 at 3. A description of what would happen to donated embryos begins on page 1 of the Consent Form under “Procedure.” Document 1 at 1-2.

We note that although HUES 1-28 are not eligible for administrative review under section II(A) of the NIH Guidelines (*see* 74 Fed. Reg. at 32174), these cell lines meet all criteria set forth therein save two. Harvard cannot document compliance with section II(A)3a, concerning the alternative disposition options that were actually available at all the IVF clinics that became involved in the donation process, (*see id.*) and II(A)3c, concerning whether each such clinic had a policy in place that a patient’s decision whether to donate embryos for research would influence the quality of care provided. *Id.* Because the embryos that resulted in HUES 1-28 may have been donated from any of a number of clinics, and because there are no remaining links that would allow us to identify from which specific clinic the embryos that resulted in a given hESC line were donated, it is impossible to backtrack and document the other disposition options that were actually available at a given clinic. However, we do know that the Collaborating IVF Clinic, where presumably most of the donations originated, presented all patients with excess frozen embryos with the following options. A couple could have: used the embryos themselves to attempt pregnancy; continued to store the embryos at the clinic; transported the embryos to another clinic or storage facility; had the embryos discarded; or donated the embryos to research. The Collaborating IVF Clinic did not then, and does not now, routinely discuss donation of embryos to another couple as an option. As to the question of differing care offered depending on a patient’s willingness to participate in research, we have

⁵ The Proposal was provided to IRB staff in October 2000 as supplemental information about the Protocol.

been told that this is not something about which clinics usually have written policies. This is a given in the health care field.

HUES 1-28 meets all of the other criteria of section II(A), however, as follows: The donated embryos were created using *in vitro* fertilization for reproductive purposes and were no longer needed for this purpose. Document 2 at 2 and 3; Document 3 at 4. They were donated by individuals who sought reproductive treatment and who gave voluntary written consent for the embryos to be used for research purposes, Document 1, Document 2 at 2 and 3. No payments, cash or in kind, were offered to the donors. Document 3 at 4, Document 2 at 3. There was a clear separation between the prospective donors' decisions to create human embryos for reproductive purposes and the prospective donor(s)'s decision to donate human embryos for research purposes. Specifically, decisions related to the creation of human embryos for reproductive purposes were made free from the influence of researchers proposing to derive or utilize hESCs in research, in that the physicians responsible for reproductive clinical care and the researchers deriving and/or proposing to utilize hESCs were not the same persons, Document 3 at 4. Moreover, consent for the donation was made at the time of donation and after treatment was completed. *Id.* In addition, donors were informed that they retained the right to withdraw consent for the donation of the embryo until the point at which the isolated cells were cultured. Document 1 at 3. The Consent Form explained: that the embryos would be used to derive hESCs for research; what would happen to the embryos in the derivation process; that hESCs derived from the embryos might be kept for many years; that the donation was made without any restriction or direction regarding the individual(s) who may receive medical benefit, such as who may be a recipient of cell transplants; that the research was not intended to provide direct medical benefit to the donor(s); and that the results of research may have commercial potential, but that the donor(s) would not receive financial or any other benefits from any such commercial development. Document 1. Finally, donors were informed that identifying information about them would not be available outside the study. *Id.* at 3. (In fact, no identifying information about the donors went to the Harvard researchers. Document 2 at 3.)

Some Additional Information for Consideration by the Working Group

Waiver of consent for broad use and wide distribution of HUES 1-28

The Consent Form explained that the Protocol was designed as a study of “embryonic development of endoderm with a focus on pancreatic formation.” Document 1 at 1. Donors were informed that the embryos were to be used to derive stem cells for this study. *Id.* at 1-2. While the Consent Form for the study noted that the results “will advance scientific and medical knowledge of human embryonic development,” and that the cell lines “may be used, at some future time, for human transplantation research,” *id.* at 2, the Consent Form mentioned only the research contemplated at that time by the PI.

HUES 1-17 were first reported in the *New England Journal of Medicine* in March 2004.⁶ Immediately after, other researchers began to request that Harvard share the lines with them. Harvard determined that despite the specificity of the Consent Form, the material being requested, which was transformed from the material donated and not identifiable to any donor, could be distributed to other scientists without restriction.

When the NAS Guidelines were issued in 2005, the PI decided it would be preferable to revise the Protocol and Consent Form to meet its new requirements. As part of this first substantive overhaul of the

⁶ Cowan CA, Klimanskaya I, McMahon J, Atienza J, Witmyer J, Zucker JP, Wang S, Morton CC, McMahon AP, Powers D, Melton DA. Derivation of embryonic stem-cell lines from human blastocytes. *N Engl J Med.* 2004 Mar 25; 350(13):1353-6.

Protocol and Consent Form, the PI decided to engage the IRB on the distribution issue. He submitted a cell line banking and distribution protocol that would involve the IRB's oversight of stem cell distribution on an ongoing basis. At the time only HUES 1-28 existed. As part of this banking protocol, the PI asked the IRB to approve Harvard's distribution of HUES 1-28 without restricting their use to pancreatic-related research only. (Embryos donated after this 2005 revision of the Protocol were used for derivation of hESCs with the expectation that the lines might be widely distributed under the banking protocol and the revised consent forms explicitly reflect this possibility.)

The IRB decided that the distribution of HUES 1-28 to the scientific community without restriction on use could be approved without obtaining any additional or updated informed consent from those who had donated excess embryos under the original Protocol. The IRB noted in its deliberation that stem cell research had advanced so quickly that the full range of uses of these lines could not have been foreseen when the original Protocol was begun. Another key factor in the IRB's deliberations was its knowledge of the way in which the identities of the donors were protected. The researchers had devised a double-coding mechanism that meant that no identifying information about donors would come to Harvard from the Collaborating IVF Clinic and assured that there would be no readily ascertainable way to link a HUES line with a donor. Applying the waiver of informed consent factors at 45 C.F.R. § 46.116(d), the IRB determined that: the research involved no more than minimal risk to subjects in that all medical procedures were long finished; granting the waiver would not pose a physical, social or privacy risk to any subject; the research could not practicably be carried out without the waiver because re-contacting donors would not only be impractical given the absence of identifying information, but also inconsistent with the Consent Form, which stated that donors "will not receive any information regarding subsequent testing on the embryo or the derived stem cells," Document 1 at 2; and attempting to re-contact donors could itself pose psychological and privacy risks to the donors. Moreover, as the IRB noted, these donors had granted explicit consent for derivation of stem cell lines from their excess embryos, as well as use of those lines for biomedical research with the overall intent of advancing scientific and medical knowledge of human embryonic development. This group of subjects was self-defined, by their consent to the original study, as a group of people who did not object to research uses of human stem cells. This analysis by the IRB is summarized in a letter Harvard uses as part of its process for distributing HUES lines, and is Document 7 in this submission.

Harvard has continued to distribute HUES 1-28 without restriction.⁷ For those institutions requesting stem cell lines that have questions about the provenance of the lines, Harvard provides a written explanation of the issue concerning the Consent Form and the IRB's waiver of informed consent process. Document 7 is the current version of that explanation.⁸

An IRB approval lapse

During the time HUES 1-28 were derived, there was a lapse between IRB approvals that bears on HUES 18-28. Specifically, the Protocol was renewed and received IRB approval on October 9, 2002 for a one-year period to expire on October 8, 2003. The next review of the Protocol by the IRB, however, did not take place until June 29, 2004, leaving a gap between the time the continuing review should have occurred and the time it actually occurred. The PI and the IRB each contributed to this lapse; the IRB was

⁷ HUES 1-17 have been the most widely distributed.

⁸ Earlier iterations of this explanation came from Harvard's Office of Technology Development, which handles the paperwork necessary to transfer the lines to recipient scientists. Eventually it was decided that the explanation should be summarized directly by Harvard's IRB.

using a new database program that sent a renewal notice to the PI that he did not see, and the database did not flag the fact that the IRB had not received the required renewal application.

When this oversight was discovered, the PI submitted the renewal application to the IRB in June 2004. The application stated that no changes were proposed for the Consent Form or Protocol since the prior renewal, apart from a change in several of the persons listed as research assistants. On June 29, 2004 the IRB approved the renewal application as submitted by the PI for a one-year period ending June 28, 2005.

During the period in which the approval had lapsed, research work continued on the Protocol. In May 2006, a newly hired research coordinator realized that research work had proceeded during the lapse. The issue was immediately reported to the IRB. The lab reported to the IRB that HUES 17-28 were derived during the lapse. It also reported that a single cell line, HUES 25, apparently originated from donors who had been consented during the lapse.

In considering the import of this information the IRB took note that there had been no changes to the Consent Form or to any IRB approved procedures used by the lab to guarantee donor confidentiality between the IRB approvals. Hence, the IRB concluded that even if the IRB's review had occurred at the scheduled time, nothing would have been different in terms of the Consent Form or the confidentiality protections afforded to the donating couples. As to HUES 25, the IRB considered whether to require re-consent. The IRB rejected this because, even if this had been possible given the double-coding confidentiality procedures, the exercise would have served no useful purpose because a donor couple would have been asked to sign a consent form identical to the one that they had signed already. Consequently, the IRB concluded that although the incident was regrettable, there was no reason to discard or restrict the lines derived during the lapse, including HUES 25.

The conclusion drawn above was supported by a review undertaken at the time of the responses of the Office for Human Research Protection ("OHRP") to noncompliance by IRBs in the performance of continuing review. During 2005 and 2006, a total of thirteen institutions received compliance letters from OHRP identifying, among other deficiencies, the failure of the institution's IRB to conduct continuing review of research on an annual basis. In all of these situations, OHRP concluded that the IRB should have suspended the enrollment of new subjects until such time as it had completed its re-review, but this action was unavailable to the Harvard IRB because the lapse was no longer in effect at the time the issue was reported to it. In no instance did OHRP require that subjects be re-consented in order to continue to participate in research activities, or require a Principal Investigator to delete or destroy research data or specimens obtained from such subjects. In each case, including one of protracted delay on the part of the reviewing IRB, OHRP's sole remedy was to require the institution to submit a corrective action plan to OHRP to ensure that the problem did not happen again.

The matter was reported to the University's Provost, to whom the IRB reported at the time. Among the reforms he mandated were that the IRB would seek accreditation, and that it change its policies to require expiration date-stamps on consent forms. The IRB instituted these and other changes and obtained accreditation from the Association for the Accreditation of Human Research Protection Programs (AAHRPP) in June 2008.

* * *

We thank you for this opportunity to have HUES 1-28 listed as human embryonic stem cell lines eligible for federally supported research.



HARVARD UNIVERSITY
Office of the Provost

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Professor of Pathology at Harvard Medical School
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t.617.384.9508
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October 1, 2009

NIH Stem Cell Registry:

I hereby certify that the statements in the Request for Human Embryonic Stem Cell Line to be Approved for Use in NIH Funded Research (NIH Form 2890), submitted by Genevieve Saphier, Associate Director for Research Policy and Compliance in the Department of Stem Cell and Regenerative Biology at Harvard University, are true, complete and accurate to the best of my knowledge.

I further confirm that that I have the authority and/or rights pertaining to the human embryonic stem cell lines identified in item 6 of the form to make this request for NIH review and determination of eligibility for use in NIH funded research. Any and all restrictions on the use of the stem cell line are clearly and completely identified in item 8 of the form.

In accordance with Section II.B. of the NIH Guidelines, I hereby assure that the embryos from which the cell lines identified in item 6 of the form were derived were donated prior to July 7, 2009, and the embryos: 1) were created using *in vitro* fertilization for reproductive purposes and were no longer needed for this purpose; and 2) were donated by donor(s) who gave voluntary written consent for the human embryos to be used for research purposes. The applicant is advised that the Working Group of the Advisory Committee to the NIH Director will consider submitted materials taking into account the principles articulated in Section II(A) of the NIH Guidelines for Human Stem Cell Research, 45 CFR 46 Subpart A, and the following points to consider: during the informed consent process, including written and oral communications, whether the donor(s) were: (1) informed of other available options pertaining to the use of embryos; (2) offered any inducements for the donation of the embryos; and (3) informed about what would happen to the embryos after the donation for research.

I acknowledge that I have read, understood, and agreed to the information provided on the form, including the Instructions for completing the form, and the Certification, Authority and Assurance provided above.

David Korn, M.D.
Vice Provost for Research
Harvard University

Document 1

Consent to Donate Human Embryos and Embryonic Cells for Research

Name of Female Donor _____ Identification # _____

Name of Male Donor _____

Title of Research Protocol: Isolation of Human Embryonic Stem Cells

Principal Investigators Names: [Name] _____, Ph.D., Scientific Director, [IVF clinic]
Douglas A. Melton, Ph.D., Professor, Harvard University
Andrew McMahon, Ph.D., Professor, Harvard University

Protocol Number:

PURPOSE OF STUDY:

This study is a research project designed to establish human embryonic stem cell (hES) lines. These cells will be used to study the embryonic development of endoderm with a focus on pancreatic formation. The long-term goal is to create human pancreatic islets that contain β cells, the cells that produce insulin, for transplantation into diabetics.

IIA3e(i)

SUBJECT SELECTION:

Before agreeing to participate in this research study, it is important that you read and understand the explanation of the proposed procedure. This consent describes the purpose, procedure, potential benefits and potential risks of the study you are being asked to participate in. It also describes the alternate procedure that is available to you. No guarantees or assurances can be made as to the results of the study.

PROCEDURE:

Your care and medications during your IVF cycle have been no different from patients who do not participate in the study. There have been no changes in the egg retrieval procedure or in assessment or culture of your embryos.

The embryos donated will not be transferred to a woman's uterus, will not survive the human pluripotent stem cell derivation process, and will be handled respectfully, as is appropriate for all human tissue used in research.

This study will isolate cells from a portion of 5 day old frozen and thawed embryos called the inner cell mass. These inner cell mass cells (a small group of cells inside the embryo) do not have the capability of becoming embryos on their own; they will be cultured as pluripotent

IIA3e(ii)

IIIB

stem cells (stem cells). These stem cells also cannot become fully developed embryos, but they do have the capacity to differentiate into a wide variety of cell types. The researchers will attempt to get them to differentiate into pancreas cells that secrete insulin. The cells may be used, at some future time, for human transplantation research. The derived cells and/or cell lines, with all identifiers removed, may be kept for many years

IIA3e(iii)

If human transplantation research using cells developed from this protocol is conducted, there can be no restriction or direction from the donors regarding the individual(s) who may be the recipient(s) of transplanted cells

IIA3e(iv)

RISKS AND DISCOMFORTS:

By agreeing to participate in this study, you will have no greater risks or discomforts than are normally associated with an IVF cycle.

You should be aware that once identifiers are removed from the cell lines, there will be no way for you to request that your donated materials be withdrawn from the research protocol. You may wish to reflect upon the permanence of this decision, and to discuss it with Dr. [Name] or others involved in the consent process.

BENEFITS:

There will be no specific benefit to you by participating in this study. The results of this study will advance scientific and medical knowledge of human embryonic development. Donors will not receive any information regarding subsequent testing on the embryo or the derived stem cells. The donated material may have commercial potential, the donor(s) will not receive financial or any other benefits from any such future commercial development.

IIA3e(v)

IIA3e(vi)

ALTERNATIVE PROCEDURES:

If you choose not to participate, your embryos will be discarded according to the usual protocol at [IVF clinic].

IIA3a

IIB

COST/PAYMENT:

The costs involved in the study are part of your routine medical care and therefore there will be no cost for participation nor will there be any payment required.

IIA3b

IIB

WITHDRAWAL FROM THE STUDY:

You may withdraw from the study at any time up to the point at which the isolated cells are cultured. All identifiers associated with the embryos will be removed prior to the derivation of the stem cells. After this point, there will be no identification of the cells with the donors.

IIA3d(iii)

IIA3e(vii)

CONFIDENTIALITY:

You have a right to privacy and the investigators on this study will take all reasonable measures to protect the confidentiality of your records. Your name and any other information which might identify you will not appear in any presentation or publication resulting from this study. Your name and any other information which might identify you will not be available to any person or group other than the investigators of this study.

CONSENT:

I have read the previous page(s) of the consent form and the investigator has explained the details of the study. I understand that I am free to ask additional questions.

If I wish additional information regarding this research and my rights as a research subject, or if I believe I have been harmed by this study, I may contact [Name], Ph.D. at *IVF clinic*.

I understand that participation in this study is voluntary and I may refuse to participate or may discontinue participation without penalty, loss of benefits, or prejudice to the quality of care that I will receive.

IIA3c

I acknowledge that no guarantees have been made to me regarding the results of the treatment involved in this study, and I consent to participate in the study and have been given a copy of this form.

Signature of Female Donor

Date

Signature of Male Donor

Date

These donors have been given the opportunity to read this consent form and to ask questions before signing, and have been given a copy.

| PRINT INVESTIGATOR'S NAME | SIGNATURE OF INVESTIGATOR (OR DESIGNEE) | DATE |
|---------------------------|---|------|
|---------------------------|---|------|

Document 2

HARVARD UNIVERSITY

FACULTY OF ARTS AND SCIENCES

Request for Approval of Human Subjects Research

Please type, or print legibly with black pen. You may follow this format on separate sheets or use additional pages if necessary.

FROM: (name, campus address)

Doug Melton, Dept of Molecular and Cellular Biology, Harvard University, 7 Divinity Ave,
Cambridge

TELEPHONE: 495-1812

E-MAIL : dmelton@biohp.harvard.edu

PROJECT TITLE: Isolation of human embryonic stem cells

ANTICIPATED FUNDING SOURCE: (include grant or contract number if known)

Howard Hughes Medical Institute and Juvenile Diabetes Foundation

FACULTY SPONSOR'S NAME: (for non-faculty applicants)

SPONSOR'S ADDRESS:

DURATION OF ENTIRE PROJECT:

from ___ASAP to ___years hence.

APPROVAL REQUESTED FOR: (maximum one year; must be renewed annually)

from ___ASAP___ to ___one year hence.

1. Please give a brief summary of the purpose of the research, in non-technical language.

We propose to establish human embryonic stem cell (hES) lines in collaboration with [IVF Clinic] of [City], an in vitro fertilization clinic. These cells will be used to study the embryonic development of endoderm with a focus on pancreatic formation. The long-term goal is to create human pancreatic islets that contain β cells, the cells that produce insulin, for transplantation into diabetics.

2. Give details of procedures that relate to subjects' participation

(a) How are subjects recruited? What inducement is offered? (Append copy of letter or advertisement or poster, if any.)

I understand the term subjects to refer to the prospective parents that have sought the services of the [IVF Clinic]. The fertilized eggs we begin with will have been previously frozen and will be obtained with the appropriate consent forms (copy attached). In vitro fertilization and freezing will be done at [IVF Clinic]. The fertilized eggs will be shipped to Harvard in the frozen state and thawed in our laboratories. The eggs will be those that would be otherwise destroyed and the parents will be informed and agree to the use of materials for this research purpose. As specified in the NIH guidelines, parental consent forms will be processed and duly recorded by [IVF Clinic].

II B

II A 1

II B

II A 1

(b) Salient characteristics of subjects--number who will participate, age range, sex, institutional affiliation, other special criteria:

Individuals interested in having IVF performed to have children. The frozen eggs to be used in our experiments will not be obtained for the purposes of our experiments (see NIH guidelines, attached) but are "left over" eggs from IVF performed at [IVF Clinic].

(c) Describe how permission has been obtained from cooperating institution(s)--school, hospital, corporation, prison, or other relevant organization. (Append letters.) NA. Is the approval of another Institutional Review Board required? No.

(d) What do subjects do, or what is done to them, or what information is gathered? (Append copies of instructions or tests or questionnaires.) How many times will observations, tests, etc., be conducted? How long will their participation take?

The subjects do not participate in the proposed experiments other than (indirectly) by supplying frozen fertilized eggs.

Following on work in mouse embryos, stem cells will be isolated from blastocysts. Fertilized eggs will be thawed and cultured for 5-7 days by which time the cells will have divided to form a blastocyst. The blastocyst will be dissected or teased apart so that cells from the inner cell mass (ICM) can be propagated in culture.

Experiments with mice have shown that embryonic stem cells (ES cells) derived by the procedure outlined above, can form any cell or tissue in the body on further development and differentiation. Comparable experiments have not been done in humans, but it is known that human ES cells can form cells representative of all three germ layers, namely ectoderm, mesoderm and endoderm.

3. List any assistant(s) who will be working with you. Cite your and their experience with this kind of research.

Various members of my lab working with mouse ES cells may assist with this work. This includes Drs. [Name], [Name], [Name], [Name], [Name], and Prof. [Name]. Graduate students include [Name] and [Name]. Technical assistance may be provided by [Name] and [Name].

4. How do you explain the research to subjects and obtain their informed

consent to participate? (If in writing, append a copy of consent form.) If subjects are minors, mentally infirm, or otherwise not legally competent to consent to participation, how is their assent obtained and from whom is proxy consent obtained? How is it made clear to subjects that they can quit the study at any time?

Please see the attached consent form.

5. Do subjects risk any harm--physical, psychological, legal, social--by participating in the research? Are the risks necessary? What safeguards do you take to minimize the risks?

The subjects risk no harm from this research. The embryos are obtained only after the subjects and their physician have agreed that the embryos are no longer needed for treatment of their infertility.

IIB

6. Are subjects deliberately deceived in any way? If so, what is the nature of the deception? Is it likely to be significant to subjects? Is there any other way to conduct the research that would not involve deception, and, if so, why have you not chosen that alternative? What explanation for the deception do you give to subjects following their participation?

No.

7. How will participation in this research benefit subjects? If subjects will be "debriefed" or receive information about the research project following its conclusion, how do you ensure the educational value of the process? (Include copies of any debriefing or educational materials)

IIA3b

Indirect benefits by advancing studies on human embryonic development.

IIB

8. How are confidentiality and/or anonymity assured? At what stage are identifiers removed from the data? If identifiers must be retained, please explain why.

Harvard research will be done without knowledge of the names of the sperm/egg donors.

IIA3e(vii)

9. Will research data (written or otherwise recorded) be destroyed at the end of the study? If not, where and in what format and for how long will they be stored? To what uses--research, demonstration, public performance, archiving--might they be put in future? How will subjects' permission for further use of their data be obtained?

No. If significant conclusions are reached, the data will be published. The subjects will not have control over, but will have access to, the data.

APPLICANT'S SIGNATURE

Douglas Melton

DATE

3 March 2000

FACULTY SPONSOR'S SIGNATURE

(for non-faculty applicants)

ATTACHMENTS:

Document 3

PROPOSAL FOR THE GENERATION OF HUMAN EMBRYONIC STEM CELLS

Investigator: Dr. Doug Melton

Collaborators: Dr. Andrew McMahon (Harvard U.) and Dr. [Name] (IVF Clinic)

Goal:

We have been interested in learning how stem cells are instructed during normal development to become pancreatic cells, in particular, the pancreatic β cell which makes insulin and which is missing in patients with type 1 diabetes. The long-term goal of our work is to generate pancreatic β cells for transplantation into diabetics. We have pursued our studies in a number of vertebrate model systems, including frogs, chicks, zebrafish and mice and will continue those studies. At the same time, it is our opinion that the answers to the questions of how human cells differentiate into tissue-specific cells should be studied with human embryonic stem (hES) cells. We therefore propose to isolate such cells from frozen preimplantation human embryos and characterize their ability to undergo differentiation in vitro.

Background:

The Melton laboratory (HHMI at Harvard University) has a longstanding interest in the developmental biology of the pancreas. We wish to understand how the pancreas normally develops and use that information to grow and develop pancreatic cells in culture. Our goals challenge us to understand the precursor, or stem cells that give rise to the pancreas. As yet, no one has isolated a stem cell that is specific for the pancreas, i.e. a pancreatic stem cell. However, studies in mice have identified embryonic stem cells, cells that can give rise to all body parts, including the pancreas.

Embryonic stem cells are cells that retain, in culture, the properties of undifferentiated embryonic cells. ES cells retain the capacity to make all the diverse cell types present in an adult organism. Under defined conditions ES cells can replicate indefinitely and maintain their pluripotent state. However, when culture conditions are appropriately modified, or when the cells are placed back in the embryo, ES cells undergo normal differentiation. Mouse ES cells have played an important role in diverse fields of modern biology including development, immunology, cancer and neurobiology. The recent demonstration that embryonic stem cells can be derived from human embryos (Thomsen et al., Science 1999) is of considerable interest because those cells, like their mouse counterparts, should be capable of forming pancreatic β cells. More generally, the human ES cells have the potential to greatly enhance our understanding of human development as well as the treatment of several human diseases.

The report by Thomsen, et al. was an exciting and potentially important scientific advance and yet there's been virtually no progress in the field for a number of reasons. The fact that the cell lines reported by Thomsen, et al. were derived with private (biotech) funding has complicated and severely restricted the distribution and analysis of human ES cells. Even if the cells reported by Thomsen were generally available, it is important to obtain a variety of human ES cells in order to fully explore their research and therapeutic potential. Research in mice has demonstrated that different mouse ES cell lines vary in their capacity to contribute to the germ

line following reintroduction into a developing embryo, their capacity for undergoing genetic recombination, and their propensity to form specific cell types during differentiation. To further complicate matters, there is presently a restriction on the derivation, but not use of, human ES cells by researchers using NIH funds. Thus, at this time, the enormous potential of the human ES cells has not been and cannot be explored by the larger research community.

Collaboration between three investigators: Melton, McMahon, and [Name] :

This proposal represents a collaboration between three principal investigators, each with a different area of expertise.

The Melton laboratory at Harvard University has been growing one human ES cell line (obtained from Drs. Nissim Benvenisty and Joseph Itskovitz working in Israel) for nearly six months. The culture conditions required for propagation and differentiation in vitro of this human ES cell line are now routine in the Melton lab. A paper (*PNAS preprint attached*) soon to be published demonstrates the effects of growth factors on this hES cell line and describes a protocol for the directed differentiation of the cells. This work has been supported by the Howard Hughes Medical Institute and the Juvenile Diabetes Foundation (and was conducted without NIH support).

The McMahon laboratory at Harvard University has considerable expertise in the derivation of mouse ES cells. The McMahon laboratory has generated approximately forty mouse ES cell lines from blastocyst cultures during the last few years. This does not represent a systematic effort to routinely derive new ES cells, but rather a specific need to generate novel ES cell lines for specific projects. The success rate presently is approximately one new cell line for every three embryos cultured (33%) comparable to the best results in published reports, and similar to those of Thomsen et al. with human embryos.

A supply of high quality fertilized eggs and preimplantation embryos is ensured by the collaboration with [Name], the Scientific and Laboratory Director at [IVF Clinic], a highly successful in vitro fertilization program in [City], Massachusetts is by some estimates the most successful IVF clinic in the U.S. [IVF Clinic] is an independent fertility treatment center that was founded in 1986. According to figures from the Society for Assisted Reproductive Technology, [IVF Clinic] performs more IVF cycles than any other clinic in the U.S. and has been responsible for the birth of more than ten thousand babies. [Name] and associates have more than ten years experience in handling frozen preimplantation human embryos and have access to a large supply of frozen embryos that are presently slated for destruction or permanent frozen storage.

Summary of the scientific method:

The published procedure for derivation of human ES cells from preimplantation embryos is very similar to that used for derivation of mouse ES cells. The only three significant differences are the embryo culture medium, the duration of blastocyst outgrowth prior to the disaggregation of the embryo and the use of immunosurgery to remove primitive endoderm cells

which may inhibit derivation of ES cell lines. Consequently, no significant problems are anticipated in generating novel human ES cell lines given a steady supply of high quality preimplantation embryos.

Embryos will be cultured to blastocyst stage in preimplantation medium, then transferred to mouse embryo fibroblast feeder layers in the presence of LIF for blastocyst outgrowth. After eight days, the inner cell mass will be isolated by immunosurgery, disaggregated, replated and ES colonies picked when of appropriate size. Cultures will be maintained at high cell density. This improves the success in maintaining pluripotent cells but requires regular passaging of cells. At each passage, (routinely one in three splits at early stages) one vial of cells will be frozen and stored in liquid nitrogen. Cultures will be maintained up to passage ten then all remaining cells will be cryopreserved.

To provide some standardization of the quality of cells the following assays on passage ten cells will be performed:

1. Immunological analysis with stage specific embryonic antigens (SSEA) and endodermal markers to assess the degree of differentiation in ES cell colonies.
2. Karyotyping (contracted out) to determine that all lines have an overtly normal chromosomal complement and to determine sex.
3. In vitro differentiation. Embryoid bodies will be generated and examined for differentiation with a number of antibody markers for differentiated cell types (e.g. nerve, muscle, cartilage, etc.)
4. hES cells will be injected, either intramuscularly or under the kidney capsule, into immuno-deficient (SCID) mice to generate teratomas and assess in vivo differentiation by histological analysis.

Early passage (passage five) hES cell lines, characterized and certified as described above, will be supplied to the ATCC for distribution. The ATCC are currently in the process of constructing a new facility to handle hES cells and have agreed, in principle, to act as distributors of the hES cells generated by this consortium. Our goal is to generate thirty hES lines in year one and another twenty lines in year two. Given an adequate supply of about two hundred frozen embryos per year and a conservative success rate of 10%-20%, this goal is well within the scope of the proposal.

Safeguards and compliance with the NIH guidelines:

The recently released NIH guidelines for work on human ES cells (*copy attached*) set out a set of conditions for obtaining frozen preimplantation embryos by informed consent. In addition, the guidelines clearly state that certain experiments; e.g. human cloning should not be

attempted. The spirit and specific provisions of the guidelines will be strictly followed in our procedures. As such, this includes, but is not limited to, the following stipulations:

Embryos will be obtained in compliance with Section II.A.2 of the NIH guidelines and documented accordingly. In particular:

1. There will be no inducement, financial or otherwise, provided to the donors of the embryos.
2. The persons responsible for obtaining donor consent was not and will not be involved in the derivation of the hES cells.
3. Frozen embryos used in the derivation procedure are excess, not required for clinical infertility procedures and were frozen and stored prior to the decision to donate the frozen embryos for research.
4. Donations of frozen embryos will be free of any conditions relating to recipients of the hES cells.
5. Donors will be given the following information:
 - Embryos will be used derive hES cells for research that may include human transplantation
 - There is no restriction or direction as to who will receive the hES cells or their derivatives
 - Information identifying the donors by name will be removed prior to hES cell derivation.
 - The hES cells and their derivatives may be stored and studied for many years.
 - Use of the hES cells may produce commercial results and/or products, from which the donor will receive no direct benefit, financial or otherwise.
 - Research using hES cells is not done for the purpose of producing a direct medical benefit to the donor.
 - The hES cells will not be transferred to a woman's uterus with the intention of producing a cloned human.
 - The frozen embryos will be dissociated and dispersed into single cells during the process used to derive hES cells.
6. Research will be conducted under the auspices of Harvard University. A registered protocol will be approved by a Harvard Institutional Review Board (IRB) for the conduct of this research. The research will be conducted in space owned or leased by Harvard.
7. No personnel, research equipment, or supplies used for this research will be supported with NIH funds. Harvard and Drs. Melton and McMahon will establish protocols to preclude the use of NIH funds on this project.
8. To the extent permitted by law, the hES cells derived under this proposal will be made available, under reasonable terms, without any reach-through rights, for dissemination to academic researchers, whether by deposit with the ATCC or otherwise.

IIA3b

IIB

IIA3d(i)

IIA1

IIB

9. *[IVF Clinic]* will receive no compensation for the embryos provided in excess of the reasonable costs for *[IVF Clinic]* services necessary to carry out the informed consent procedures, to thaw the frozen embryos, and to prepare the embryos for delivery to Dr. McMahon for derivation of hES cells. Ownership of inventions made during the derivation process and in the research using the hES cells will be determined in good faith on the basis of legal inventorship, with each inventor assigning to his/her institution, subject to any applicable laws and regulations.

Notes on donor recruitment and obtaining frozen embryos:

It should be noted that we do not propose to use cells derived from aborted fetuses. All of the embryos used in the proposed study will come from *[IVF Clinic]* clinic, from patients who have completed their infertility treatment, and whose embryos are in frozen storage. There will likely be a three-month lag time from the start of the project to the delivery of the first embryos to allow for patient contact and consent.

The requirements for the donation of embryos for research are described in detail in the Report and Recommendations of the National Bioethics Advisory Commission, Volume 1, page 72 and Appendix F. These include detailed guidelines for the separation of clinical treatment (for infertility) and the patient/donor's decision to provide supernumerary embryos for research. These recommendations will be followed in our protocol. Much of the expense incurred by *[IVF Clinic]* (see budget) covers the personnel costs necessary to ensure that all the provisions are appropriately followed.

Budget estimate for Year 1:

Personnel:

| | |
|---|---------|
| 1 Senior Research Associate (\$XXXX /yr + fringe benefits [32.1%]) | \$ XXXX |
| 2 Technicians- tissue culture (\$XXXX 'yr + fringe) | \$XXXXX |
| 1 Technician- animal work (\$XXXX 'yr + fringe) | \$ XXXX |

Supplies:

| | |
|---|---------|
| Tissue culture reagents (media, serum, LIF, etc.) | \$ XXXX |
| Sterile plasticware | \$ XXXX |
| Antibodies | \$ XXXX |
| Karotyping (\$XXX /line by contract) | \$ XXX |
| Histology (\$XXX /line by contract) | \$ XXX |

Equipment:

| | |
|--|---------|
| 2 Baker laminar flow tissue culture hoods | \$ XXXX |
| 3 Forma double bank water jacket incubators | \$ XXXX |
| 2 Leica/Zeiss inverted phase microscopes | \$ XXXX |
| 3 Wild dissecting stereo scopes (one with trinocular hood) | \$ XXXX |
| JVC video camera (KYF-70) | \$ XXXX |

| | |
|--|---------|
| Leica micromanipulator | \$ XXXX |
| One liquid nitrogen freezer | \$ XXXX |
| Waterbaths, refrigerator, freezer, pipette aids, centrifuges, etc. | \$ XXXX |
| Computer, rewritable CD, printer, FAX | \$ XXX |

Mouse costs (SCID studies and feeder layer production):

| | |
|--|---------|
| Purchase (SCID \$ ^{XX} /mouse x 200) | \$ XXXX |
| Per diem (^{XX} /cage day x 10,000 cage days) | \$ XXX |

Cost to obtain frozen embryos with consent (*IVF Clinic*):

| | |
|--------------------------------------|---------|
| Recruiting, legal and administrative | \$ XXXX |
| Embryologist (part time + fringe) | \$ XXXX |
| Supplies | \$ XXXX |

| | |
|--|-----------------|
| Total Budget Requested, Year 1: | \$XXXXXX |
|--|-----------------|

Budget estimate for Year 2:

Personnel:

| | |
|------------------------------------|----------|
| Same as year 1, 4% salary increase | \$XXXXXX |
|------------------------------------|----------|

Supplies:

| | |
|--|----------|
| | \$XXXXXX |
|--|----------|

Equipment:

| | |
|--|---------|
| Miscellaneous and replacement of small items | \$ XXXX |
|--|---------|

Mouse costs:

| | |
|--|---------|
| | \$ XXXX |
|--|---------|

Embryo associated (*IVF Clinic*):

| | |
|--|---------|
| | \$ XXXX |
|--|---------|

| | |
|--|-----------------|
| Total Budget Requested, Year 2: | \$XXXXXX |
|--|-----------------|

Explanatory notes:

Personnel costs. The senior research associate will provide technical advice and general supervision, contributing 20% of effort to this project. All other personnel will contribute 100% effort.

Equipment. Funds are requested for a fully equipped tissue culture suite with the capability for digital imaging and documentation of cultures and embryo karyotyping. The creation of a separate and devoted tissue culture room for hES cell work ensures the separation from NIH supported research.

Obtaining embryos (*IVF Clinic*). This includes costs for patient/donor recruitment, interviews and discussion of informed consent, storage of cryopreserved embryos and embryo transport to Harvard University. In addition, technical supervision of thawing embryos and growing them to the blastocyst stage (embryologist, part-time) is included in this portion of the budget.

Document 4

HARVARD UNIVERSITY
COMMITTEE ON THE USE OF HUMAN SUBJECTS IN RESEARCH

FARISH A. JENKINS, JR.
Chairman
Dean R. Gallant
Executive Officer

Science Center 123
1 Oxford Street
Cambridge, Massachusetts 02138
(617) 495-5459

REPORT OF COMMITTEE ACTION

Investigator: Douglas Melton
Project Title: Isolation of human embryonic stem cells

Funding Source: Howard Hughes Medical Institute

ACTION TAKEN

- ☐ 1 Approved as submitted ☐ 3 Approved as amended
☒ 2 Approved with conditions (see below) ☐ 4 Not approved

TYPE OF REVIEW

- ☐ Expedited Review date: 12/14/2000
☒ Full review

Conditions, comments, etc.:

Approval was contingent upon:

1. The "Risks and Discomforts" section of the consent form should mention the emotional potential of participation since, once identifiers are removed from the cell lines, there will be no way for subjects to request that their donated materials be withdrawn from the research protocol. It is important that subjects be given the opportunity to reflect upon the permanence of this decision, and be able to discuss the situation with Dr. [Name] or others involved in the consent process.
2. Members found the first paragraph under Procedure in the consent form confusing (referring as it does to events that will have already taken place) and suggested that it be rewritten to clarify that participation will involve no new procedures for the donors.
3. For stem cells derived using this protocol to be usable by NIH funded researchers in future, in accordance with the NBAC recommendations and the NIH guidelines, the third paragraph of "Procedure" should mention that if human transplantation research using cells developed from this protocol is conducted, there can be no restriction or direction from the donors regarding the individual(s) who may be the recipient(s) of transplanted cells.

Period of approval: 12/14/00 - 12/13/01

If project extends beyond approval period,
renewal application must be submitted by: 11/13/01

ALL APPROVALS ARE SUBJECT TO THE FOLLOWING CONDITIONS:

1. Changes in procedures that may significantly affect subjects or significantly alter the experience of subjects' participation must be reported to the Committee for approval in advance. Minor changes may be approvable by expedited review; major changes may require action at an assembled Committee meeting.
2. Continuation of subject participation beyond the approval period requires renewal of approval by separate application. *It is the investigator's responsibility to submit renewal requests in a timely fashion.*
3. Should there be reason to think that a subject is suffering or has suffered any harm, anticipated or not, as a result of participation, the investigator must suspend the research and report to the Committee. The research shall not resume without Committee approval.
4. Expedited approvals are granted with the understanding that the Committee may impose additional conditions after review at a convened meeting.
5. Approval confirms that the project as proposed is not in conflict with the Committee's rules and regulations, but it does not imply endorsement or sponsorship by the University. Although investigators may indicate their position at Harvard, they shall not represent that the research is sponsored by the University or a department within the University except by explicit arrangement with appropriate administrative authorities.

for the Committee,



Dean R. Gallant
Executive Officer

Date: 12/19/2000

cc: Andrew McMahon

Document 5



THE COMMONWEALTH OF MASSACHUSETTS
MIDDLESEX DISTRICT ATTORNEY
40 THORNDIKE STREET CAMBRIDGE MA 02141

MARTHA COAKLEY
DISTRICT ATTORNEY

December 19, 2001

Tel: 617-679-6500
Fax: 617-225-0871

BY FAX (617-495-5079)
AND BY FIRST-CLASS MAIL

Robert W. Iuliano
Deputy General Counsel
Office of the General Counsel
Harvard University
Holyoke Center, Suite 980
1350 Massachusetts Avenue
Cambridge, MA 02138-3834

Re: Research Protocol Filed Pursuant to G.L. c. 112, § 12J

Dear Mr. Iuliano:

This office has received your filing dated November 20, 2001, pursuant to G.L. c. 112, § 12J(a)(VI), including: your cover letter; the protocol of research proposed by Professor Douglas A. Melton of the Harvard University Department of Molecular and Cellular Biology; and the 4-page Report on Approval of Protocol by the Harvard University Faculty of Arts and Sciences Institutional Review Board.

Based on a review of that filing and analysis of the statute, G.L. c. 112, § 12J, there are no "reasonable grounds" to believe that research conducted pursuant to the protocol would violate the provisions of the statute. G.L. c. 112, § 12J(b)(I). This conclusion is based at least in part on the three reasons set forth in the Institutional Review Board's Report on Approval of Protocol.

Sincerely,

Marguerite T. Grant
Senior Appellate Counsel
617-679-6542

cc: Martha Coakley, District Attorney



Document 6

HARVARD UNIVERSITY

STEM CELL RESEARCH COMMITTEE

Richard M. Losick, Chair
Biological Laboratories 3023
(617) 495-4905 / fax 496-4642
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CAMBRIDGE, MASSACHUSETTS 02138

Dcan R. Gallant, Secretary
Science Center 123
(617) 495-2628 / fax 495-1210
drg@fas.harvard.edu

27 March 2002

Douglas Melton
Thomas Dudley Cabot Professor
of the Natural Sciences
Fairchild 465

Dear Doug,

I am writing to confirm, formally, the Stem Cell Research Committee's approval of the human embryonic stem cell projects described in your memorandum to me of October 26th, 2001. To summarize our actions: the Committee met twice to discuss these projects. At our first meeting, on December 21st, we voted to approve Project 1, the isolation of hES cell lines, as well as that part of Project 2 involving *in vitro* manipulations. We requested further information about the *in vivo* part of Project 2, and your response was circulated to Committee members prior to the second meeting on February 11th, at which you and Andy McMahon were present to answer questions from the Committee. Following lengthy discussion the Committee voted to approve the *in vivo* portion of Project 2.

Members of the Committee appreciated your willingness to report to us on the progress of the research, including important developments such as a significant uptake of human cells in the animal embryo. We will be interested to learn from you of any developments that might assist in our review of your projects, and other embryonic stem cell research at Harvard, but will not require such reporting as a condition of this approval.

The Committee's approval for both Project 1 and the two arms of Project 2 will extend through February 10, 2003. At that point (and at yearly intervals thereafter, so long as the research continues) we will ask that you request reapproval of the research, and include a brief summary of any results you have obtained. If, prior to that time, you anticipate modifications to the experimental protocols, or new avenues of research involving hES cells, that differ significantly from these two projects, we ask that you submit a description of the new protocol(s) for Committee review and approval.

Good luck with your endeavors.

Sincerely yours,



Richard M. Losick

c: A. McMahon
Provost Steven Hyman
SCRC members

Document 7

HARVARD UNIVERSITY
COMMITTEE ON THE USE OF HUMAN SUBJECTS IN RESEARCH

Alfonso Caramazza
Chairman
Dean Gallant
Executive Officer

50 Church St., 5th Floor
Cambridge, Massachusetts 02138
(617) 495-2628

17 July 2008

CUHS Statement regarding distribution of HUES Lines 1 - 28

Due to queries from researchers and their institutions regarding IRB-approved uses of these lines, we have prepared this document describing the actions of the Committee on the Use of Human Subjects.

The Committee met on August 1, 2005 to consider a request of Harvard faculty member, Douglas Melton, the Principal Investigator under the protocol entitled "Establishment of tissue bank for human embryonic stem cells," to approve the creation of a stem cell tissue bank, and to allow for the deposit in that bank and distribution therefrom of stem cell lines derived under the original protocol. The Principal Investigator's application was for a waiver of consent as to the deposit and distribution of these stem cell lines, which are coded without any identifiers linking them to subjects as HUES 1 - 28. The Committee considered the question with the assistance of Barbara F. Mishkin, a noted legal expert on human subjects research from the law firm of Hogan & Hartson.

As part of its consideration of the matter, the Committee first noted that at the time the original pancreatic study was approved in 2000, the full scope and potential use of the stem cells derived under the protocol was not anticipated. The Committee then went on to consider the application under the waiver of informed consent requirements set forth in 45 CFR 46.116(d). The Committee approved the inclusion of HUES lines 1-28 in a tissue bank, granting a waiver of informed consent, having determined that:

(1) the research involved no more than minimal risk

All medical procedures involving the donors had already occurred prior to the beginning of the research, and the proposed inclusion of derived hESC lines in a tissue bank would occur only after identifiers had been removed from the cells. Therefore, the Committee determined that there were no physical or privacy risks to the subjects in granting the waiver.

(2) the waiver or alteration will not adversely affect the rights and welfare of the subjects

The creation of a tissue bank and deposit and distribution of HUES 1-28 would present no physical, social, or privacy risks to subjects, for the reasons stated above. In addition, the Committee noted that the subjects had already been informed of, and had already given their permission for, the potential use of the stem cell lines in biomedical research, including transplantation research. The overall intent of the research as explained in the consent form was to advance scientific and medical knowledge of human embryonic development. The consent form stated that donor-subjects would not receive information about subsequent testing on embryos or derived stem cells. The consent form also indicated that subjects' donated material might have commercial potential, but that the donor(s) would not receive financial or any other benefits from

such future commercial development. Thus the Committee could see no adverse effect on any benefits to which subjects would otherwise be entitled for the stem cells lines to be made available under the tissue bank's criteria for further scientific and medical research.

(3) the research could not practicably be carried out without the waiver or alteration

Given the absence of links to individual donors, it was not only impracticable to attempt to obtain consent from the donors whose cells had contributed to a specific line in order for the cell lines to be distributed for scientific use, but would have been inconsistent with the representation in the consent form that the donors "will not receive any information regarding subsequent testing on the embryo or the derived stem cells." The Committee also considered that re-contacting the donors could itself pose psychological and privacy risks to some subjects.

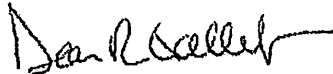
(4) whenever appropriate, the subjects will be provided with additional pertinent information after participation.

Removal of identifiers meant that donor-subjects could not be identified or linked to a derived cell line, and thus provision of additional information to former subjects was neither possible nor relevant.

Thus the Committee determined that the necessary conditions for waiver or alteration per 45 CFR 46.116(d) were met, and there would be no requirement to re-consent subjects to allow HUES lines 1-28 to be deposited in, and distributed from, a tissue bank.

In summary, the stem cell lines may be used, in their current form, for any legitimate scientific purpose, including DNA analyses.

for the Committee on the Use of Human Subjects,



Dean R. Gallant



HARVARD UNIVERSITY
Office of the Provost

David Korn, MD
Vice Provost for Research
Professor of Pathology at Harvard Medical School
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November 3, 2009

Ellen L. Gadbois, Ph.D.
Office of Science Policy Analysis
Bldg 1 Room 218D
Bethesda, MD 20892-0166

RE: Requests for more information from Working Group on our application for approval for use with federal funds of HUES 1-28

Dear Dr. Gadbois:

Thank you very much for your careful review of our application for approval for use with federal funds of HUES 1-28. We will do all we can to assist the Working Group so that HUES lines 1-28 can be deemed eligible for registration and use in federally funded research. Before answering your questions, there are two issues we wish to bring to the Working Group's attention.

First, we would like to make clear, as perhaps we might not have in our submission, that the embryos from which these lines were derived were donated years ago, when the only available guidance specific to human embryonic stem cell research was the *National Institutes of Health Guidelines for Research Using Human Pluripotent Stem Cells*, 65 Fed. Reg. 51976 (Aug. 25, 2000), commonly referred to as the "Clinton Guidelines." Our research protocol and consent were very carefully crafted with these guidelines specifically in mind, and in particular with Section II.A.2.c of said Guidelines, which states, "To ensure that human embryos donated for research were in excess of the clinical need of the individuals seeking fertility treatment and to allow potential donors time between the creation of the embryos for fertility treatment and the decision to donate for research purposes, only frozen human embryos should have been used to derive human pluripotent stem cells. In addition, individuals undergoing fertility treatment should have been approached about consent for donation of human embryos to derive pluripotent stem cells only at the time of deciding the disposition of embryos in excess of the clinical need." *Id.* at 51980.

The NIH's commentary accompanying the most recent version of the *National Institutes of Health Guidelines for Research Using Human Pluripotent Stem Cells*, 74 Fed. Reg. 32170 (July 7, 2007), hereafter referred to as the "New Guidelines," addressed the subject of "grandfathering" existing lines by pointing out that the process of ACD review would allow existing lines to be evaluated for eligibility for federal funding *in the context of the guidelines available at the time of derivation* (*italics added*). Specifically, the response to public comments provides that "the NIH is also cognizant that in the more than a decade between the discovery of hESCs and today, many lines were

derived consistent with ethical standards and/or guidelines developed by various states, countries, and other entities such as the International Society for Stem Cell Research (ISSCR) and the National Academy of Sciences (NAS) . . . It is important to recognize that the principles of ethical research, e.g., voluntary informed consent to participation, have not varied in this time period, but the requirements for implementation and procedural safeguards employed to demonstrate compliance have evolved. . . This Working Group will not undertake a *de novo* evaluation of ethical standards, but will consider the materials submitted in light of the principles and points to consider in the Guidelines, as well as 45 CFR Part 46 Subpart A.” *Id.* at 32170.

Our protocol was designed in 2000-01, and pre-dated the NAS and ISSCR stem cell guidelines. We followed the only then-existing stem cell research specific guidelines and believe that careful examination of the materials we have submitted will reflect this.

Second, we made choices in the early days of this research that now limit our ability to obtain documentation that was not contemporaneously gathered by us. The research proposal took several years of review before it was approved. During those years the federal government made it clear that it was not supportive of hESC research, and we were made very much aware that there were many interest groups zealously opposed to this research on religious or moral grounds. Among the things we considered was that our researchers had received death threats. Thus, we took steps beyond those usually taken to protect donor **confidentiality** to construct a program that would provide donor **anonymity**. One of our goals was to make certain that we would not have in our possession information that would identify donors or that could be used to link donors to the stem cell lines. We did not desire a situation in which donors could ever be traced or targeted by opposition groups. Thus, the information we have retained about the process is all we have access to, and we cannot now go back and find ways to link paperwork that we never had to donors or embryos.

To respond specifically to the requests made by the Working Group:

- ***Your application includes lines derived from embryos from a variety of different IVF clinics. Is it possible to determine whether any particular line came from an embryo donated at a particular IVF clinic? Did the “Collaborating Clinics” retain records of the source clinics of the embryos?***

There are no links that would allow us to identify from which specific clinic the embryos that resulted in a given hESC line were donated.

- ***If available, the Working Group would like to review representative clinical consent forms from the IVF clinics providing reproductive treatment to the embryo donor(s) for lines HUES1-28.***

We have asked the Collaborating Clinic for a copy of a treatment consent form in use in the year 2000. We do not know when or whether the Collaborating Clinic will agree, or indeed be able to provide us with such an exemplar. The Working Group should recognize that the IVF treatment afforded to donors may have occurred at various times much earlier than 2000, further compounding the difficulty inherent in meeting this request.

Moreover, the donation of embryos to our research only ever occurred after a couple's treatment cycle was completed. We know this because we only accepted frozen embryos that were "obtained only after the subjects and their physician [had] agreed that the embryos [were] no longer needed for treatment of their fertility." Protocol, Document 2 at 3. The treatment consent was never considered part of the research protocol and was never a document that was reviewed as part of the donation process because the administration of that treatment consent would have pre-dated any research involvement. We were careful to design our protocol in a way that separated the research from the treatment so that the clinical treatment of the patients (including their consent to treatment) was concluded before any conversation occurred regarding donation.

Given these several facts, we would appreciate your help in understanding why the Working Group is seeking to review the treatment consents of embryo donors. Although we are unable to provide the treatment consents for the originating clinics, and may never be able to provide even an example of a treatment consent from the Collaborating Clinic, we do appreciate that the Working Group may be concerned about whether donors were informed of other available options pertaining to the use of the embryos. Although the options presented to a particular couple at a particular clinic may be presented in varying language, the universe of disposition options available to IVF patients for their excess frozen embryos is limited to:

1. continuing to store the embryos (either at the IVF clinic, or at another facility),
2. using the embryos themselves to attempt pregnancy,
3. donating the embryos to another couple for them to use to attempt pregnancy,
4. discarding the embryos, and
5. donating the embryos to research.

As the New Guidelines acknowledge, not all clinics make all of these options available to their patients. Even for lines that will be derived after issuance of the New Guidelines, the NIH does not prescribe that all possible disposition options must be communicated to all IVF patients, but rather that "All options available in the health care facility where treatment was sought . . . were explained to the individual(s) who sought reproductive treatment." New Guidelines, 74 Fed. Reg. at 32174.

Though later versions of our research consent form (those developed after the NAS Guidelines of 2005) lay out these options explicitly, at the time the Protocol was approved, it was seen as unnecessary to do so. Because the clinics had no incentive to persuade donors to participate in the research, we believed it reasonable to conclude that the research option merely would have been added to the disposition options the clinics usually offered.

- ***Further description of the timing of consent for reproductive services and the timing of consent for the donation of the embryos for research for a typical donor represented in lines HUES1-28.***

As stated above, our protocol was designed in compliance with the Clinton Guidelines, and with the following specifically in mind: "To ensure that human embryos donated for research were in excess of the clinical need of the individuals seeking fertility treatment and to allow potential donors time between the creation of the embryos for fertility treatment and the decision to donate for research purposes, only frozen human embryos should have been used

to derive human pluripotent stem cells. In addition, individuals undergoing fertility treatment should have been approached about consent for donation of human embryos to derive pluripotent stem cells only at the time of deciding the disposition of embryos in excess of the clinical need." Clinton Guidelines, 65 Fed. Reg. at 51980.

Under the Protocol, consent for embryo donation was always obtained at the time of donation, and never in advance of the creation of the embryos. Consent for reproductive services is always obtained before any treatment is given (i.e., before an IVF cycle is begun). Consent for embryo donation was only obtained from couples who had completed their IVF cycle and had excess frozen embryos. Under the design of the Protocol, couples who had not yet completed their IVF cycle would not have been asked to participate in the research, and we have no reason to believe the Protocol was not followed. Consent for treatment would, therefore, have to be separated from consent for donation by at least the duration of the IVF cycle (4-8 weeks, depending on the treatment regime) and could have been separated by multiple years. Because we can no longer link HUES 1-28 to individual donors, it is impossible for us to estimate an average amount of time that passed between the treatment consent and the donation consent.

- ***Any further information about who did the initial consenting at the other clinics for embryo donation and how the consent processes at the other clinics and the Collaborating Clinic were coordinated.***

In most cases, the other clinics were not involved in consenting donors for donation. With respect to all donations except for the situation described below, the consent for donation was obtained by staff at the Collaborating Clinic. Patients interested in donating embryos from other clinics were told they could contact the Collaborating Clinic, and if they did make contact, trained members of the Embryology team at the Collaborating Clinic would explain the research study to potential donor couples. If the couples were still interested after talking with the Collaborating Clinic staff, they would be mailed a copy of the consent form, which they would sign and mail back to the Collaborating Clinic. Once the Collaborating Clinic received the signed consent form, they would coordinate with the other clinic or embryo storage facility to receive shipment of the embryos.

There was one clinic that preferred to conduct the informed consent conversations with its patients. The laboratory director at that clinic was trained by the Collaborating Clinic staff regarding the inclusion and exclusion criteria for donation of embryos to the research and consenting the donors. This laboratory director consented potential donors who had been treated at her clinic.

- ***Further description of any role of the Scientific Director at the "Collaborating IVF Clinic" with regards to:***
 - ***obtaining consent for donation of the embryos,***

The Scientific Director provided oversight to the Collaborating Clinic personnel who conducted the consent conversations, but did not directly conduct these conversations himself. He was, however, available to answer any questions that potential donors might have.

- *subsequent involvement in research using the human embryonic cell lines derived from the donated embryos*

Because at the time the Melton lab had little experience thawing embryos, the Scientific Director oversaw this process. Also, the Scientific Director and Dr. Melton consulted about the design of experiments, with the Scientific Director contributing his knowledge about human embryology – subjects such as optimal embryo culture conditions. The Scientific Director had no direct involvement, other than thawing, in deriving stem cell lines or in using them.

- *Further description of the financial relationship between the Collaborating IVF Clinic and Harvard University (including intellectual property) and any authorship agreements.*

The financial relationship between Harvard University and the Collaborating Clinic was laid out in detail in a collaboration agreement (the “Collaboration Agreement”) signed by Harvard, the Collaborating Clinic, and the Howard Hughes Medical Institute. (Dr. Melton was and remains a Hughes Investigator.) The Collaboration Agreement stated that the Collaborating Clinic was to be reimbursed for its reasonable costs to obtain all necessary consents for the donation of the embryos and to thaw, prepare for derivation of hES cells, and transfer the embryos to Harvard. This was the extent of the financial arrangement, which was intended to ensure that the Collaborating Clinic had no financial incentive to recruit donors.

As to intellectual property rights, the Collaborating Agreement defined “Invention” as including patentable work related to hES cells and their derivation arising out of the research collaboration. The Collaborating Agreement then provided that all Inventions (whether made solely by employees of one party or jointly by employees of two or more parties) would not be patented and would be placed in the public domain through publication. That is, it was the intent of the parties that neither the processes used to derive human embryonic stem cells nor the cells themselves would be patented, but should be placed in the public domain through publication.

As to publication/authorship, the Collaborating Agreement provided that the parties would jointly publish results, although each reserved the right alone to publish information and data generated in the course of the research project. Further, the Agreement provided that authorship of articles reporting the results of research would be determined in accordance with academic standards and custom, and that proper acknowledgement would be made for the contributions of each party to the research results being published.

We thank you again for this opportunity to have HUES 1-28 listed as human embryonic stem cell lines eligible for federally supported research.

Sincerely,

A handwritten signature in black ink, appearing to read "David Korn, M.D.", with a stylized, cursive script.

David Korn, MD
Vice Provost for Research, Harvard University
Professor of Pathology, Harvard Medical School

From: [Korn, Dr David](#)
To: [Gadbois, Ellen \(NIH/OD\) \[E\]](#)
Cc: [Saphier, Genevieve](#); [Lopez, Diane](#); [Kandziolka, Laura J](#)
Subject: Harvard's Response to Your Request for Further Information
Date: Friday, November 13, 2009 5:22:48 PM
Attachments: [Clinic attestation 111309.doc](#)

Dear Dr. Gadbois

I am writing on behalf of Harvard University in response to your request of November 12 for additional information regarding our submission for consideration of registration of 28 HUES human embryonic stem cell lines. Harvard attests that the attached document is an attestation from the Medical Director of Harvard's Collaborating IVF Clinic that addresses the menu of choices that were offered to Clinic patients who sought IVF treatment, and whose "excess" stored embryos were ultimately donated to Harvard University for human embryonic stem cell research that resulted in the generation of HUES lines 1-28.

I hope that this response will prove acceptable to the review committee and help to advance our request for registration.

Naturally, I will be happy to answer any further questions that arise.

Cordially,

David Korn, M. D.
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I attest, as the medical director of the IVF clinic that collaborated with Harvard University in the donation of excess frozen embryos for stem cell research, that all patients of our clinic are informed of the available disposition options for their frozen embryos. At our clinic, these include: using the embryos themselves to attempt pregnancy; continuing to store the embryos at the clinic; transporting the embryos to another clinic or storage facility; discarding the embryos; or donating the embryos to research. To the best of my knowledge, this menu of options, or one very similar to it (I cannot be certain that we offered the option of donation for research in the 1990s), was offered to our patients during the time period that frozen embryos in excess of those needed for their own reproductive purposes were considered for donation to Harvard University for stem cell research.

Though I think it likely that this menu of options is consistent with that offered to patients of other clinics who donated embryos through us to Harvard University, I have no direct knowledge of the policies and procedures of other IVF clinics.